surface proteins and activate the complement cascade. Complement proteins, specifically C3, are fixed to the virus. This opsonized virus can then bind to complement receptors on macrophages, lymphocytes, or follicular dendritic cells. The virus can thereby infect these cells through this pathway.

Although in vitro antibody-dependent enhancement has been reported for a number of viruses, it has been difficult to show enhanced infections in vivo. The best documented example is dengue virus where clinically substantial dengue hemorrhagic fever and dengue shock syndrome occur in the presence of enhancing antibodies. Indeed, the severity of disease was correlated to the levels of enhancing antibodies. In vitro and in vivo antibody-dependent enhancement has been described for a number of animal viruses as well and is an important concern in veterinary vaccine development. This phenomenon of antibody-dependent enhancement and the viruses for which it has been observed were recently reviewed.

Infection by the human immunodeficiency virus (HIV) is accompanied by potent host immune responses against the virus. Early in HIV infection, patients have measurable neutralizing antibody titers, antibodies that can lyse HIV-infected cells, and cytotoxic lymphocytes that can kill virus-containing $CD4^+$ lymphocytes. In theory, all of these responses should combine to eradicate HIV infection in the host. Nevertheless, despite these immune responses, persons infected by HIV continue to progress through the full gamut of HIV-induced disease and finally die of complications of the acquired immunodeficiency syndrome (AIDS). This fact led investigators to search for possible adverse immune responses that may complicate the host response to HIV infection. In 1987, investigators first reported antibody-dependent enhancement of HIV infection in vitro. Later reports indicated that this enhancement could occur through both the complement and Fc receptor-mediated mechanisms. It has been difficult, however, to show a clear clinical role for enhancing antibodies in HIV infection. The best data on the role of enhancing antibodies in HIV-induced disease may come from studies using animals. Two separate studies using rhesus macaques and the simian immunodeficiency virus (SIV) have shown that enhancing antibody levels increase throughout the disease course and peak before death of the animals from AIDS. This animal model is arguably the best one for HIV infection. It has also been shown that chimpanzees infected with HIV have antibodies that can enhance HIV infection in vitro. Nevertheless, these antibody levels appear to decline the longer the chimpanzee is infected. Chimpanzees do not go on to have AIDS, nor do they become clinically ill following HIV inoculation.

Formidable difficulties are involved in studying enhancing antibodies in a disease that takes years to become clinically apparent. The average length of time for patients to progress to AIDS is 12 years. Even if high levels of enhancing antibodies resulted in a change in disease progression from 12 years to 8 years, it would require a huge clinical sample.

The greatest concerns to practitioners involve the theoretic risk of antibody-dependent enhancement in HIV infection. To date, no one has yet proved that in vivo enhancing antibodies of HIV infection do or do not play a role in disease progression. Circumstantial evidence such as disease progression despite potent immune responses to HIV and vaccine failure in animals suggests that in vivo antibody-dependent enhancement may be a reality. Can the theoretic risks be ignored, thus placing thousands of lives at risk if mass inoculation is given to persons in HIV-endemic areas with vaccines that produce antibody-dependent enhancement?

Despite the protestations of some investigators, mass immunization of many persons in high-risk areas of the world with vaccines that have proved ineffective in animals may occur. If these immunized patients show an increased susceptibility to HIV infection, then enhancing antibodies are important. If such studies progress as planned, we can only hope that antibody-dependent enhancement of HIV infection, unlike dengue virus, will be only an in vitro artifact. At this time, possible risks outweigh any demonstrated benefits to the putative AIDS vaccine being tested. A quick cure is not in sight for this pandemic.

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Iron Deficiency

STUDIES HAVE ASSOCIATED iron deficiency with epithelial changes, impaired immune response, abnormal temperature regulation, deranged catecholamine metabolism, and abnormal physical activity independent of any anemic effects. More recent studies have shown behavioral changes, reduced exercise tolerance, decreased levels of the neurotransmitter γ-aminobutyric acid in brain, altered lipid metabolism, and abnormal myelination of the central nervous system. Many of these diverse effects are reversible with iron therapy, whereas others may be partially reversible or permanent.

Of particular interest has been the effect of iron deficiency on the central nervous system independent of any concomitant anemia. Some parts of the brain contain considerable quantities of iron; iron is required for the activity of enzymes such as hydroxylases and monoamine oxidases that are important in brain function. Iron also influences myelination by its effects on lipid metabolism, causing changes in the fatty acid composition and content of lipids in both peripheral circulation and brain. Brain sphingolipid of iron-deficient animals has shown a pronounced reduction of fatty monoenoic acids such as ω9,C18:1 and ω9,C24:1. Iron supplementation of deficient animals results in at least a partial correction of these deficits. Iron is an essential requirement for desaturase enzyme function in liver, and iron deprivation might be expected to depress comparable desaturase activity in the fetal and neonatal brain. Observations to date show that iron deficiency is associated with dramatic changes in the fatty acid composition of myelin-specific lipids such as cerebroside.

Extensive studies done in children and adults indicate disturbances of behavior and cognitive factors with iron deficiency alone. In one study series, infants having iron deficiency with and without anemia tended to score lower in the Bayley scale of mental development than those without sideropenia. A clear sensory disturbance of taste has been well documented in adults with iron deficiency with and without

anemia eventuating in pica. A striking form of this disorder is a compulsive craving for ice, or pagophagia. The craving can be corrected with iron therapy before the correction of any accompanying anemia. The links between any neurologic clinical manifestations of iron deficiency and demonstrated biochemical abnormalities are tenuous, but the information to date would justify renewed clinical concern about the status of iron nutrition in people and especially in young children or neonates.

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Molecular Techniques

ONCE A FRINGE, highly specialized, and esoteric area largely relegated to basic research, DNA-based diagnostic techniques are now applied routinely to anatomic and clinical pathology tests. These techniques involve the manipulation of DNA for the purpose of detecting nucleotide sequences of clinical or forensic importance. These sequences are typically detected by hybridization (specific nucleotide base-pairing) with fragments of DNA or RNA (called probes) containing the complementary sequence. With the isolation (or cloning) of an ever-expanding array of disease-specific genes and sequences, a process now accelerating rapidly under the impetus of the human genome project, the list of available molecular pathology tests is growing weekly.

Specific molecular reagents now exist for the laboratory diagnosis of more than 100 genetic diseases, a myriad of infectious pathogens, tumor markers of diagnostic or prognostic importance, and the generation of individual-specific DNA fragment patterns (fingerprints) for paternity testing, forensic identification, twin zygosity determination, and transplant engraftment confirmation. The target for these hybridization reagents may be crude extracts of blood, tissue or body fluid DNA (the dot blot), electrophoresed fragments of restriction endonuclease-digested genomic DNA (the Southern blot), routinely processed tissue sections mounted on glass slides (in situ hybridization), or even metaphase or interphase chromosome spreads (molecular cytogenetics). Applications include the rapid identification of infectious agents (such as mycobacteria) without the need for lengthy microbial culture and biochemical species characterization procedures, prenatal diagnosis and carrier testing for genetic disorders (such as cystic fibrosis), and the diagnosis and staging of neoplastic processes by the detection of characteristic gene rearrangements and alterations in oncogenes and tumor suppressor genes (such as lymphoma and adenocarcinoma of the colon).

Spanning all of these applications, the use of the polymerase chain reaction continues to revolutionize the field, enhancing both the sensitivity and specificity of molecular techniques by many orders of magnitude. By replicating discrete regions of genomic DNA with DNA polymerase, the technique amplifies exponentially the amount of analyte available for study, allowing testing of the most minute clinical specimens (even single cells) while enabling one to home in on the specific region or target sequence of interest. It greatly simplifies the search for mutations associated with genetic diseases and malignant neoplasms and for microbial pathogens present in sparse infections (for example, human immunodeficiency virus in blood) and in dead or fixed specimens that are no longer culturable. The polymerase chain reaction can also be applied to DNA fingerprinting (by amplifying hypervariable regions of the human genome), allowing the identification of trace or degraded forensic specimens. By enabling genetic analysis of single cells, it has opened the way for preimplantation and even preconception diagnosis.

Like any new technology, molecular pathology has some drawbacks. Many of these tests are technically difficult, time-consuming, and expensive, requiring specialized resources and personnel often unavailable outside large academic centers and reference laboratories. Numerous ethical issues arise, impinging on matters such as genetic privacy, abortion, insurability and stigmatization, patent rights, and eugenics. Despite these challenges, diagnostic DNA techniques have already transformed and revitalized the practice of pathology in many exciting ways.

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